TABLE III. Species Specificity of Rabbit and Human Sera as Sources of Inhibitor *vs* C'1-Esterase Prepared from Rabbit or Human Serum.*

Inhibitor source	C'1-Esterase source	Inhibitor titer, units/ml
Rabbit serum	Rabbit	2.0
" "	Human	3.0
Human "	Rabbit	5.0
" "	Human	7.7

* Each serum was assayed against equivalent amounts of rabbit and human C'1-esterase under the usual conditions.

rabbit C'1 by the same procedure used for human C'1 (6.7). The inhibitory activity of rabbit and human sera *vs.* rabbit and human C'1-esterase was then compared (Table III). Rabbit serum was again found to have a lower titer of inhibitor than human serum even when tested against rabbit esterase. It was concluded, therefore, that at least with respect to these two species, no marked species specificity existed in the interaction of C'1-esterase and serum inhibitor.

Discussion and Summary. An assay is described for measurement of a serum inhibitor of an esterase derived from preparations of the first component of complement (C'1-esterase). Esterolysis of N-acetyl-L-tyrosine ethyl ester by C'1-esterase is inhibited instantaneously and stoichiometrically by fresh human serum. Units of C'1-esterase and of serum inhibitor are defined. The inhibitor in human serum is stable at 4° for at least 4 months, at 37° for at least 24 hr. and at 48° for at least 30 min. It is completely inactivated during 30 min. incubation at 60° and is labile at 0° at pH values below 6. The inhibitor is inactivated at −5° in the presence of methanol concentrations of 15% or greater but is recovered quantitatively in a 40% ammonium sulfate supernatant fraction. These data are being applied to purification of the inhibitor from human serum. Purification procedures and properties of the purified inhibitor will be reported elsewhere.

Monkey and guinea pig sera inhibit human C'1-esterase to about the same extent as human serum, while rabbit serum is about one-third as active. No marked species specificity exists in the interaction of inhibitor in human or rabbit serum with human or rabbit C'1-esterase. These observations are being employed on possible role of inhibitor in experimental hypersensitivity.

1. Lepow, I. H., Wurz, L., Ratnoff, O. D., Pillemer, L., *J. Immunol.*, 1954, v73, 146.

2. Lepow, I. H., Pillemer, L., *ibid.*, 1955, v75, 63.

3. Lepow, I. H., Ratnoff, O. D., Pillemer, L., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 111.

4. Becker, E. L., *J. Immunol.*, 1956, v77, 462.

5. ———, *ibid.*, 1956, v77, 469.

6. Lepow, I. H., Ratnoff, O. D., Levy, L. R., *J. Exp. Med.*, 1958, v107, 451.

7. Lepow, I. H., Ratnoff, O. D., Rosen, F. S., Pillemer, L., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 32.

8. Ratnoff, O. D., Lepow, I. H., *J. Exp. Med.*, 1957, v106, 327.

9. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., Taylor, H. L., *J.A.C.S.*, 1946, v68, 459.

Received May 22, 1959. P.S.E.B.M., 1959, v101.

Relationship of Infectious Canine Hepatitis Virus to Human Adenovirus (25035)

JACOBA G. KAPSENBERG (Introduced by D. W. Bruner)

Laboratory for Virus and Rickettsial Diseases, Nat'l Inst. of Public Health, Utrecht, Netherlands

A virus strain (Utrecht) was isolated from a fatal case of hepatitis contagiosa canis (HCC) in trypsinized dog kidney cell culture. Inclusions as described by Leader(1) were found in nuclei of these cells when stained with hematoxylin and eosin. In cross complement-fixation tests with a reference dog serum* and a reference virus strain,† the iso-

* Obtained from Dr. H. Kunst, originally received from Prof. Hjarre (Stockholm).

† Received through courtesy of Dr. J. H. Gillespie, Cornell University, Ithaca, N. Y.

lated virus was identified as a strain of hepatitis contagiosa canis (HCC) virus. A microscopic comparison of the intranuclear inclusions in dog kidney cells and similar inclusions found in adenovirus infections of our human epithelial cell lines revealed so striking a resemblance that further investigation into the relationship of the Utrecht or other HCC virus strains and the adenovirus group seemed indicated. The characteristics of the adenovirus group(2) are: 1. Cytopathogenicity for susceptible tissue culture cells. 2. Nonpathogenicity for the usual laboratory animals. 3. A complement-fixing antigen common to all members. 4. Resistance to ethyl ether. HCC virus was tested regarding these 4 points.

Materials and methods. Virus. Two strains of HCC virus were obtained, one tissue culture adapted† and the other as virulent dog liver.‡ Strain Utrecht was isolated in dog kidney cell culture in our laboratory. *Tissue culture.* Trypsin dispersed dog kidney cells were prepared according to a modification of the method described by Youngner(3) and grown in a medium consisting of Hanks' balanced salt solution with 0.5% lactalbumin hydrolysate and 5% calf serum. Cells were maintained in medium 199 with 5% calf serum. Sometimes second passage cells were used. T_1-cells(4) were grown and maintained in the same media. *Complement fixation.* Quantitative complement-fixation tests were performed in plates. Two hemolytic units of complement were used. The mixtures were incubated overnight at 4°C.

Results. 1. The cytopathogenic effect of HCC virus strain Utrecht in dog kidney cells consists in a rounding of cells, which become highly refractile. Sometimes characteristic grapelike clusters of rounded cells are formed. Finally the cells detach from the glass. With the adapted strain, the CPE of undiluted virus usually is completed within 2 to 3 days, whereas the incubation period increases with dilution of the infecting virus. After fixation with Bouin's fluid and staining with hematoxylin and eosin, the first changes in the nuclei seem to be one or more small finely granular eosinophilic masses surrounded by a clear zone. More nuclei contain one large granular eosinophilic mass, separated from the nuclear membrane by a clear zone. Often this zone is subdivided into compartments by radiating lines. The nucleoli are pushed towards the nuclear membrane or incorporated in the central mass. In the latter case they are surrounded by a clear halo. In later stages they are no longer recognizable. Sometimes the affected nuclei are enlarged.

These findings agree exactly with those described by Boyer(5) for adenovirus type 3 and 4 in HeLa cells and found with our adenovirus strains of type 3, 4 and 7 in the human kidney cell line T_1. The only difference is that HCC virus seems not to produce crystal-like structures.

2. As is known from experimental evidence (6,7) the HCC virus is not infectious for guinea pigs, rats, mice, baby mice, rabbits and chick embryos. In our experience, the Utrecht strain is somewhat toxic for suckling mice; we could not pass the virus, nor could we find histological reactions indicating an infection. The chorioallantoic of 10-day-old duck embryos was not susceptible either. The only naturally susceptible animals seem to be dogs and foxes(8).

3. In preliminary experiment, an antigen was prepared from virulent dog liver according to directions of Mansi(9). Human sera were selected regarding presence or absence of complement-fixing antibodies for adenovirus antigen. The antigen we used is made from adenovirus type 1 and 5 grown in T_1 cells. The results are shown in Table I. All sera positive with adenovirus antigen react with HCC liver antigen too.

After these promising results, antigens were made from 3 strains grown in dog kidney cell culture. Strain Rotterdam was received as a virulent dog liver suspension and passed once in tissue culture; strain Ithaca was received in its 119th dog kidney passage. The antigens were heated for one hour at 56°C. Dr. van der Veen (Tilburg) tested these antigens with paired sera from patients with adenovirus infections in complement fixation. The type isolated from their throat washings is indicated

‡ Received from Dr. O. Bosgra, Philips-Roxane, Weesp, and Dr. G. M. van Waveren, Rijksserum Inrichting, Rotterdam.

TABLE I. Comparison of C. F. Titers in Human Sera Tested with Adenovirus and HCC Liver Antigen.

Serum No.	Complement fixation titer: Adenovirus antigen	Complement fixation titer: HCC liver antigen
902	32	8
943	128	32
964	32	8
970	32	8
985	64	32
930	<4	<4
944	<4	<4
951	<4	<4
963	<4	<4
1000	<4	<4

Titers expressed as reciprocal of highest dilution giving complete fixation.

in the Table. Human control sera from cases of nonadenovirus infections were included. Table II shows the results of complement fixation tests carried out with HCC antigens and the adenovirus antigen made from adenovirus type 4 grown in HeLa cells. All sera show an almost equal rise in complement-fixing antibodies, whether they were tested with the known adenovirus antigen or each of the HCC antigens. As the sera were derived from patients infected with various adenovirus types, the HCC strains and the adenovirus types share a common C.F. antigen.

4. After treatment with 20% ethyl ether during 18 hours at 4°C, a virus suspension of strain Utrecht had the same infectious titer as the untreated control (10^{-4}).

Discussion. From these results, it seems justified to consider the virus of infectious canine hepatitis as a member of the adenovirus group.

In some of the clinical manifestations, infectious canine hepatitis also resembles adenovirus infections in man. Many exposed dogs suffer from conjunctivitis, fiery redness of the mucous membranes of the oral cavity and tonsillitis. Many show a transient corneal opacity of one or both eyes(10,11). A similar transient keratoconjunctivitis is described for adenovirus type 3(12). A keratoconjunctivitis of longer duration is observed in persons infected with adenovirus type 8(13). The association of adenoviruses with pharyngoconjunctival fever is well known(2). On the other hand, symptoms of the respiratory system are rare in dogs with infectious hepatitis. Adenoviruses in man do not show a tropism for endothelial and liver cells, as is characteristic for Rubarth's disease(10) nor is a human liver disease known with similar intranuclear inclusions. Lethality of human adenovirus infections is extremely low.

With respect to its tendency for persistence

TABLE II. Complement Fixation Titer of Sera from Patients with Adenovirus and Other Infections.

Serum patient No.		Isolated type adenovirus	Antigens: Adenovirus	Antigens: Hepatitis contagiosa canis: Utrecht	Antigens: Hepatitis contagiosa canis: Rotterdam	Antigens: Hepatitis contagiosa canis: Ithaca	Antigens: Control dog kidney
1	A*	3	5	5	10	5	0†
	C		20	40	40	40	0
2	A	4	5	10	10	10	ND
	C		40	40	80	40	0
3	A	4	10	10	10	5	0
	C		1280	320	640	1280	0
4	A	7	0	5	5	5	0
	C		40	40	40	40	0
5	A	14	10	10	10	20	0
	C		80	40	80	80	0
6	A	Control	0	0	0	0	0
	C		0	0	0	0	0
7	A	Control	0	10	10	10	0
	C	(Influenza A)	0	10	10	10	0

* A = acute phase serum. C = convalescent phase serum.
† 0 = < 1:5; ND = not done.
Titers expressed as reciprocal of highest dilution giving complete fixation.
Test by Dr. J. van der Veen.

after infection, HCC virus behaves as a member of the adenovirus group. The virus can be excreted with the urine for as long as 271 days(11). The virus was "unmasked" in noninoculated dog kidney cell cultures derived from a dog 3 months after recovery from an infection(14). In the same way, adenovirus was detected in cultures made from human adenoid tissue.

As far as we know, HCC virus is not pathogenic for man. The claim of inapparent human infections made by some authors(15) was based on the finding of C.F. antibodies in human sera. This should be reviewed in the light of the relationship between HCC strains and adenoviruses.

It is not yet known if the virus of infectious canine hepatitis is a new member of the adenovirus group or related to one of the human or simian types. Dr. van der Veen informed us that the prototype adenovirus strains of types 1-17 could not be neutralized by our guinea pig antiserum to the Utrecht strain of HCC virus at a serum dilution of 1:4. This serum had a neutralizing titer of 1:320 for the homologous strain. Typing experiments are in progress and will be published. The host range and virulence of HCC virus points to a separate position among the members of the adenovirus group. Future experience will show whether the animal host range is appropriate for the further division of adenoviruses into human, simian and canine subgroups.

Summary. 1) The virus of infectious canine hepatitis was shown to share the 4 main properties of the adenovirus group, including common complement-fixing antigen. 2) The relationship between the disease of the dog and human adenovirus infections is discussed.

1. Leader, R. W., *Am. J. Vet. Res.*, 1958, v19, 152.
2. Rowe, W. P., Huebner, R. J., Bell, J. A., *Ann. N. Y. Acad. Sci.*, 1957, v67, 255.
3. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
4. van der Veen, J., Bots, L., Mes, A., *Arch. Ges. Virusf.*, 1958, v8, 230.
5. Boyer, G. S., Leuchtenberger, C., Ginsberg, H. S., *J. Exp. Med.*, 1957, v105, 195.
6. Cabasso, V. J., Stebbins, M. R., Norton, T. W., Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 239.
7. Fieldsteel, A. H., Emery, J. B., *ibid.*, 1954, v86, 819.
8. Evans, C. A., Dowell, M., Green, R. G., *J. Infect. Dis.*, 1950, v86, 1.
9. Mansi, W., *J. Comp. Path.*, 1955, v65, 291.
10. Rubarth, S., *Acta Path. Microb. Scan.*, 1947, Suppl. 69.
11. Witter, R. E., Jensen, H. E., Baker, J. A., *J.A.V.M.A.*, 1954, v124, 214.
12. Fowle, A. M. C., Cockeram, A., Ormsby, H. L., *Am. J. Ophthalmol.*, 1955, v40, 180.
13. Jawetz, E., Kimura, S., Nicholas, A., Thygeson, P., Hanna, L., *Science*, 1955, v122, 1190.
14. Bolin, V. S., Jarnevic, N., Austin, J. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 414.
15. Delage, B., Martin, L. A., *Arch. Int. Pasteur du Maroc.*, 1955, v5, 60.

Received May 28, 1959. P.S.E.B.M., 1959, v101.

Human Blood Group Substance B and *Escherichia coli* 086. (25036)

LOUIS H. MUSCHEL AND ETSUKO OSAWA
(Introduced by M. E. Webster)
Walter Reed Army Inst. of Research, Washington, D.C.

Many investigators have considered selective influences of ABO blood groups on disease conditions. A marked selection against heterozygous fetuses and infants occurs, for example, when the mother is group O and the father is group A or group B(1). Moreover, Race and Sanger(2) have postulated that it is unlikely that the relationship between blood groups and disease would work only through maternal-foetal incompatibilities. A significant association has been found between the ABO groups and duodenal ulcer, carcinoma of stomach, and diabetes mellitus(3). Also, in infectious diseases, individuals of blood group B appear to possess a slightly greater degree of natural resistance to poliomyelitis(4).